



Faculty of Resource Science and Technology

**Chitosan Derived Nanoparticles –based Sensing Probes for Detection of
Japanese Encephalitis (JE) Antigen**

Lai Huat Choi

**Doctor of Philosophy
2021**

Chitosan Derived Nanoparticles – based Sensing Probes for Detection of Japanese Encephalitis (JE) Antigen

Lai Huat Choi

A thesis submitted

In fulfillment of the requirements for the degree of Doctor of Philosophy

(Physical Chemistry)

Faculty of Resource Science and Technology

UNIVERSITI MALAYSIA SARAWAK

2021

DECLARATION

I declare that the work in this thesis was carried out in accordance with the regulations of Universiti Malaysia Sarawak. Except where due acknowledgements have been made, the work is that of the author alone. The thesis has not been accepted for any degree and is not concurrently submitted in candidature of any other degree.

.....

Signature

Name: Lai Huat Choi

Matric No: 14010046

Faculty of Resource Science and Technology

Universiti Malaysia Sarawak

Date : 17 February 2020

ACKNOWLEDGEMENT

I would like to express my deepest gratitude and thanks to my research mentor, Associate Professor Dr. Chin Suk Fun for her support, help, enthusiasm, guidance and invaluable advice for providing the opportunity for me to carry out this research. The preparation of this research would not have been possible without her unconditional assistance, her endless encouragement and meticulous guidance. My warmest thanks also go to the only member of my advisory committee, Professor Dr. Pang Suh Cem for his technical inputs and encouragement throughout the course of my study.

I wish to acknowledge Mr. Safri, Assistant Scientific Officer and Madam Ting Woei, Scientific Officer at Faculty of Resource Science and Technology for their helpful technical assistance particularly SEM and TEM micrographs presented in this dissertation were beautifully taken. Nevertheless, I would like to thank the staffs and laboratory assistants of Faculty of Resource Science and Technology, University Malaysia Sarawak for their help throughout the research. This study is supported by the Ministry of Education Malaysia (KPM) rendered through the award of the Exploratory Research Grant Scheme (ERGS/STG05(01)/1005/2013(02) and the financial support from My PhD for the author throughout the degree of Doctor of Philosophy is gratefully acknowledge.

Finally, this research owes its completion to the constant encouragement and perseverance of family members, as well as the support of many friends especially Ying Ying, Lih Shan, Shao Chien, Ain, Lionel, Angeline and all the lab mates, your caring and supportive gestures as well as the joyful moment together are greatly appreciated and missed.

ABSTRACT

Japanese encephalitis virus (JEV), a mosquito-transmitted flavivirus that will lead to neurological diseases and death in humans in extreme cases. Up to date, there are currently no specific anti-viral medications and curative treatment for JEV. Therefore, early, effective and accurate diagnosis of JEV infection by using a point-of-care, simple, rapid and economical biosensor is very critical for the prevention and control of JEV outbreaks. A biosensor is an analytical device containing biocatalyst and a transducer for point-of-care screening which converts any diseases or harmful biological events to detectable signals. Designing and fabricating biosensors with a higher rate of reaction and sensitivity has to become popular among researchers due to the emergence of nanotechnology. In recent decades, carbon nanoparticles (CNPs) have attracted numerous researchers to study their nanoscience and nanotechnology potential comparing to other nanoparticles due to their special characteristics especially their optical and electrochemical properties which turn them into potential candidates for bioimaging, biosensing, drug delivery, and photodynamic applications and most importantly CNPs are easier to synthesize, environmental friendly and low cost. In this study, based on the electrical conductivity performance of CNPs derived from the various types of precursor characterized by using cyclic voltammetry, (CV) and electrochemical impedance spectroscopy, (EIS), chitosan CNPs with the smallest size and performed highest electrical conductivity are selected for further modification in order to fabricate a higher rate of reaction and sensitivity of electrochemical biosensor. Carbon nanoparticles modified on screen-printed carbon electrode (CNPs-SPCE) electrochemical biosensor strip has been successfully fabricated by immobilized JEV antibody onto the surfaces of carbon nanoparticles through amide bonds formed between amino groups of CNPs and carboxylic groups of JEV antibody. The analytical performance of the CNPs-

SPCE electrochemical biosensor strip was characterized using CV and EIS. CNPs-SPCE electrochemical biosensor strip exhibited a linear detection range of $1 - 20 \text{ ngmL}^{-1}$ with a low limit of detection (LOD) of 0.36 ngmL^{-1} (at $S/N = 3$) for JEV, detection sensitivity was 0.024 ngmL^{-1} for JEV and analysis results were obtainable within 10 minutes. In order to enhance the sensitivity and selectivity of the CNPs-SPCE electrochemical biosensor strip, the addition of Gold nanoparticles (AuNPs) onto CNPs to produce the hybrid Gold-Carbon nanoparticles (Au-CNPs) which provides a higher effective surface area, catalytic activity and electrical conductivity has been successfully fabricated. The analytical performance of hybrid Gold-Carbon nanoparticles modified on screen-printed carbon electrode (Au-CNPs-SPCE) electrochemical biosensor strips was characterized using CV and EIS. Au-CNPs-SPCE electrochemical biosensor strip exhibited a linear detection range of $1 - 20 \text{ ngmL}^{-1}$ with an extremely low LOD of 0.29 ngmL^{-1} (at $S/N = 3$) for JEV, detection sensitivity was 0.04 ngmL^{-1} for JEV and responding time is 10 minutes. The potential clinical application of this Au-CNPs-SPCE electrochemical biosensor strip was demonstrated by the detection of JEV in human serum while the selectivity of this biosensor strip was also proven by using Dengue antigen. In conclusion, virus infections can cause serious diseases to humans and animals as certain viruses could spread rapidly within a very short period. Hence, the fabrication of this accurate and fast biosensor for early detection of viruses is often crucial for clinical diagnosis and therapy.

Keywords: Carbon nanoparticles (CNPs), Japanese encephalitis virus (JEV), electrochemical biosensors, hybrid gold-carbon nanoparticles (Au-CNPs), chitosan

Alat Pengesan Sensor Berasaskan Nano-Zarah Karbon Diperolehi dari Kitosan untuk Mengesan Antigen Japanese Encephalitis (JE)

ABSTRAK

Virus Japanese encephalitis adalah sejenis flavivirus yang berjangkit melalui gigitan nyamuk boleh membawa kepada penyakit neurologi dan juga kematian jikalau jangkitan itu menjadi semakin teruk. Sehingga kini, tiada ubat anti-virus dan rawatan khas untuk JEV. Oleh itu, diagnosis yang lebih awal, cepat dan tepat bagi jangkitan JEV adalah sangat penting untuk mencegah dan mengawal wabak JEV merebak. Biosensor adalah peranti analisis yang mengandungi bio-mangkin dan transduser yang boleh mengesan apa-apa penyakit atau aktiviti biologi yang berbahaya dan mengubah kepada isyarat yang dapat dikenalpasti. Reka bentuk dan fabrikasi biosensor dengan kadar tindak balas dan sensitiviti yang tinggi adalah sangat popular di kalangan para penyelidik sejak kemunculan bidang nanoteknologi. Kebelakangan ini, CNPs telah menarik perhatian ramai penyelidik untuk mengkaji potensi dan keberkesanan kegunaannya dalam bidang nano-sains dan nanoteknologi berbanding dengan nano-zarah yang lain. Ini adalah kerana ciri khusus mereka terutamanya sifat optik dan sifat elektrokimia yang menjadikan CNPs sebagai calon pilihan utama untuk bioimaging, biosensing dan yang paling penting adalah CNPs mesra alam, proses penyediaan yang lebih mudah dan murah. Dalam kajian ini, berdasarkan kepada prestasi kekonduksian elektrik untuk pelbagai jenis CNPs yang diuji dengan menggunakan CV dan EIS, kitosan CNPs dengan saiz terkecil dan kekonduksian elektrik tertinggi dipilih untuk kajian selanjutnya supaya untuk menghasilkan biosensor yang mempunyai kadar tindak balas dan sensitiviti yang tinggi. Fabrikasi biosensor elektrokimia CNPs-SPCE berjaya disediakan dengan pembentukan ikatan amida antara kumpulan berfungsi $-NH_2$ dari CNPs dan kumpulan berfungsi $COOH$ dari antibodi JEV. Prestasi biosensor

elektrokimia CNPs-SPCE diuji melalui CV dan EIS. Strip biosensor elektrokimia CNPs-SPCE mempamerkan julat pengesanan linear antara 1 hingga 20 ngmL⁻¹ dan mempunyai LOD yang rendah iaitu 0.36 ngmL⁻¹ (pada $S / N = 3$), kepekaan pengesanan untuk JEV ialah 0.024 ngmL⁻¹ dan keputusan analisis boleh didapati dalam masa 10 minit. Untuk meningkatkan kepekaan dan kepelbagaian biosensor elektrokimia CNPs-SPCE, penambahan AuNPs keatas CNPs untuk menghasilkan hibrid Emas-Karbon nano-zarzh (Au-CNPs) yang mempunyai jumlah luas permukaan yang lebih tinggi, aktiviti mangkin, kekonduksian elektrik dan bio-keserasian yang jauh lebih berkesan. Prestasi biosensor elektrokimia Au-CNPs-SPCE mempamerkan julat pengesanan linear 1 hingga 20 ngmL⁻¹ dengan LOD yang sangat rendah iaitu 0.29 ngmL⁻¹ (pada $S / N = 3$) kepekaan pengesanan ialah 0.04 ngmL⁻¹ untuk JEV dan masa yang bertindak balas adalah 10 minit. Potensi penggunaan klinikal untuk biosensor elektrokimia Au-CNPs-SPCE ini ditunjukkan oleh pengesanan JEV dalam serum manusia dan kajian pemilihan virus turut dibuktikan dengan menggunakan antigen denggi. Kesimpulannya, jangkitan virus boleh membawa penyakit yang serius kepada manusia dan haiwan disebabkan terdapat sebahagian virus tersebut boleh menyebar dengan cepat dalam tempoh yang sangat singkat. Oleh itu, pengesanan jangkitan virus dengan lebih awal, berkesan dan cepat adalah sangat penting untuk diagnosis dan rawatan di klinik.

Kata kunci: Nano-zarah karbon (CNPs), virus Japanese encephalitis (JEV), biosensor elektrokimia, hibrid emas-karbon nano-zarah (Au-CNPs), kitosan

TABLE OF CONTENTS

	Page
DECLARATION	i
ACKNOWLEDGEMENT	ii
ABSTRACT	iii
<i>ABSTRAK</i>	v
TABLE OF CONTENTS	vii
LIST OF TABLES	xiv
LIST OF FIGURES	xv
LIST OF ABBREVIATIONS	xix
CHAPTER 1: INTRODUCTION	1
1.1 Background and Problem Statement	1
1.2 Objectives	9
1.3 Chapter Summary	9
CHAPTER 2: LITERATURE REVIEW	12
2.1 Biosensor	12
2.1.1 Biocatalytic Sensor	12
2.1.1.1 Glucose Sensors	13
2.1.1.2 Xanthine Sensors	13
2.1.1.3 Lactate Sensors	14

2.1.2	Bio-affinity Sensors	15
2.1.2.1	Immunoassays and Immunosensors	15
2.1.2.2	DNA Hybridization Biosensors	16
2.2	Carbon-Based Nanostructures	16
2.2.1	Fullerenes	17
2.2.2	Carbon Nanotubes	18
2.2.3	Graphene	18
2.2.4	Nanodiamonds	19
2.2.5	Nanofibers	20
2.2.6	Nanoparticles	20
2.2.6.1	Preparation of Carbon Nanoparticles	21
2.2.6.2	Properties of Carbon Nanoparticles	22
2.2.6.3	Application of Carbon Nanoparticles as Electrochemical Sensor	23
2.2.6.4	Used of Chitosan Nanoparticles as Electrochemical Sensors	24
2.3	Electrochemical Detection	25
2.3.1	Voltammetry and Amperometry	25
2.3.2	Impedance	26
2.4	Electrodes	27
2.4.1	Conventional Electrodes	28
2.4.2	Screen-Printed Carbon Electrodes (SPCE)	29

2.5	Immobilization Procedure	30
2.5.1	Used Direct Immobilization On Electrode Surface	31
2.5.2	Use Other Solid Support as Carrier	31
2.6	Japanese Encephalitis Virus (JEV)	32
2.6.1	Overview of Japanese Encephalitis Cases	32
2.6.2	The Conventional Diagnostic Methods for JEV Detection	32
2.6.2.1	Enzyme-linked Immunosorbent Assays (ELISA)	33
2.6.2.2	Plaque Reduction Neutralisation Test	33
2.6.2.3	Reverse Transcription Polymerase Chain Reaction (RT-PCR)	34
2.6.2.4	Virus Isolation	35
2.6.3	Electrochemical Immunosensors for JEV Detection	35
2.6.3.1	Application of CNPs modified SPCE Electrochemical Biosensor Strip	35
2.6.3.2	Application of Au-CNPs modified SPCE Electrochemical Biosensor Strip	36
CHAPTER 3: SYNTHESIS AND CHARACTERISATION OF VARIOUS CARBON-BASED NANOPARTICLES (CNPs) FOR BIOSENSING APPLICATION		38
3.1	Introduction	38
3.2	Materials and Methods	41
3.2.1	Chemicals	41
3.2.2	Synthesis of Preformed Nanoparticles from Different Types of Precursor	42

3.2.3	Synthesis of Carbon Nanoparticles (CNPs) from Different Types of Precursor	42
3.2.4	Instrumentation	42
3.2.5	Electrochemical Characterization	43
3.3	Results and Discussion	43
3.3.1	Size and Morphology of Carbon Nanoparticles	43
3.3.2	Electrochemical Characterization of Carbon Nanoparticles	46
3.3.2.1	Cyclic Voltammetry (CV)	46
3.3.2.2	Electrochemical Impedance Spectroscopy (EIS)	48
3.3.3	Relationship between Size of CNPs and its Electrical Conductivity	49
3.3.4	Optimization for the Temperature on Preparation of Chitosan CNPs	51
3.4	Conclusions	58
 CHAPTER 4: CARBON NANOPARTICLES (CNPs) BASED ELECTROCHEMICAL BIOSENSOR STRIP FOR DETECTION OF JAPANESE ENCEPHALITIS VIRUS (JEV)		 60
4.1	Introduction	60
4.2	Materials and Methods	62
4.2.1	Chemicals	62
4.2.2	Synthesis of Carbon Nanoparticles (CNPs)	63
4.2.3	Isolation of JEV	63

4.2.4	Activation of the JEV Antibody and Immobilizing on the Surface of CNPs	64
4.2.5	Instrumentation	64
4.2.6	Preparation of CNPs-SPCE Electrochemical Biosensor Strip	65
4.2.7	Detection of JEV antigens	65
4.3	Results and Discussion	67
4.3.1	Morphology of Chitosan Nanoparticles and Carbon Nanoparticles (CNPs)	67
4.3.2	Characterization of Immobilized JEV Antibody onto CNPs	68
4.3.3	Electrochemical Characterization of the Immunosensor	69
4.3.3.1	Cyclic Voltammetry (CV)	69
4.3.3.2	Electrochemical Impedance Spectroscopy (EIS)	70
4.3.4	Specificity, Selectivity, and Capability	72
4.3.5	The LOD of <i>Japanese encephalitis</i> virus (JEV) based on EIS	73
4.3.6	Response Time of the Immunosensor	74
4.4	Conclusion	76
 CHAPTER 5: FABRICATION OF HYBRID GOLD-CARBON NANOPARTICLES (Au-CNPs) BY SELF ASSEMBLY METHOD FOR JAPANESE ENCEPHALITIS VIRUS (JEV) ELECTROCHEMICAL BIOSENSOR		 78
5.1	Introduction	78
5.2	Materials and Methods	80
5.2.1	Chemicals	80

5.2.2	Preparation of Hybrid Gold-Carbon Nanoparticles (Au-CNPs)	81
5.2.3	Characterisation of Hybrid Gold-Carbon Nanoparticles (Au-CNPs)	81
5.2.4	Optimization of Experimental Parameters	82
5.2.5	Activation of the JEV antibody and Immobilizing on Surface of Au10-CNPs	82
5.2.6	Fabrication of Hybrid Au10-CNPs Modified SPCE Electrochemical Biosensor	83
5.2.7	Detection of JEV antigens	83
5.3	Results and Discussion	85
5.3.1	Characterization of Hybrid Gold-Carbon nanoparticles (Au-CNPs)	85
5.3.1.1	Ultraviolet-visible Spectrophotometer (UV-Vis)	85
5.3.1.2	Transmission Electron Microscope (TEM)	86
5.3.1.3	Field Emission Scanning Electron Microscope (FESEM) and Energy Dispersive X-ray (EDX) spectroscopy	88
5.3.1.4	Cyclic Voltammetry (CV)	90
5.3.1.5	Electron Impedance Spectroscopy (EIS)	91
5.3.2	Optimization of Experimental Parameters	92
5.3.2.1	Effect for Amount of AuNPs Loading on CNPs	92
5.3.2.2	Effect of Electrolyte Temperature	94
5.3.2.3	Response Time of the Immunosensor	95
5.3.3	Specificity, Selectivity and Capability	97

5.3.4	Sensitivity of Immobilised Hybrid Gold-Carbon Nanoparticles Modified on SPCE Electrochemical Biosensor Strips	98
5.4	Conclusion	99
	CHAPTER 6: CONCLUSION AND RECOMMENDATIONS	101
6.1	Concluding Remarks	101
6.2	Recommendations for Future Works	103
	REFERENCES	105
	APPENDICES	134

LIST OF TABLES

	Page
Table 2.1 Function of electrode (Hayat & Marty, 2014).	27

LIST OF FIGURES

	Page
Figure 1.1 Schematic diagram of the working principle of the electrochemical biosensor (Dhull et al., 2013).	4
Figure 2.1 Carbon nanostructures with different dimensions (Mohamed, 2017).	17
Figure 2.2 Image of a simple Nyquist plot.	27
Figure 2.3 Image of SPCE, (a) Reference electrode, (b) Working electrode, and (c) Counter electrode.	30
Figure 2.4 Fabrication of sensitive detection of AFP. (Asadian et al., 2019)	37
Figure 3.1 FESEM micrograph of CNPs from different types of precursor (A) carboxymethyl cellulose, (B) starch, (C) fibrous cellulose, (D) citric acid, (E) ascorbic acid and (F) chitosan	45
Figure 3.2 Bar chart showed the particles size and error bar (calculated from standard deviation of particles size for 50 discrete and spherical nanoparticles) of CNPs synthesized from different types of precursor.	46
Figure 3.3 CV of bare SPCE strip and CNPs from different types of precursor.	47
Figure 3.4 Nyquist plots of bare SPCE strip and CNPs from different types of precursor. The inset shows the Randle Equivalent Circuit.	49
Figure 3.5 Scatter plot showed the relationship between the different size of CNPs from different types of precursor versus electron-transfer resistance (Ret). (Y error bar is calculated from standard deviation of particles size for 50 discrete and spherical NPs)	50
Figure 3.6 Photo for the chitosan CNPs prepared through various reflux temperature.	53
Figure 3.7 FTIR spectra of chitosan CNPs prepared through various reflux temperature.	54
Figure 3.8 SEM micrograph of the particles prepared through various reflux temperature.	55
Figure 3.9 Scatter plot showed the relationship between the different size of particles from different types of precursor versus electron-transfer resistance (Ret).	56
Figure 3.10 XRD pattern of chitosan CNPs reflux at 180 °C.	56

Figure 3.11	CV of bare SPCE strip and chitosan CNPs prepared through various reflux temperature.	57
Figure 3.12	EIS of bare SPCE strip and chitosan CNPs prepared through various reflux temperature.	58
Figure 4.1	Schematic diagram of preparation processes employed on the SPCE strip for fabricating electrochemical biosensor for the detection of JEV (Lai et al., 2017).	66
Figure 4.2	Scanning electron micrographs of (A) chitosan nanoparticles, (B) carbon nanoparticles (CNPs) in x10000 and (C) carbon nanoparticles (CNPs) in x20000 used for the immobilization of JEV antibody.	67
Figure 4.3	FTIR spectra of (a) carbon nanoparticles (CNPs), and (b) JEV antibody immobilized onto carbon nanoparticles.	69
Figure 4.4	CV of (i) bare SPCE strip; (ii) CNPs modified SPCE strip; (iii) JEV antibody immobilized CNPs on SPCE strip; (iv) BSA blocked/JEV antibody immobilized CNPs on SPCE strip; (v) BSA blocked/JEV antibody immobilized CNPs on SPCE strip in the presence of JEV	70
Figure 4.5	Nyquist plots of (i) bare SPCE strip; (ii) CNPs modified SPCE strip; (iii) JEV antibody immobilized CNPs on SPCE strip; (iv) BSA blocked/JEV antibody immobilized CNPs on SPCE strip; (v) BSA blocked/JEV antibody immobilized CNPs on SPCE strip in the presence of JEV; (vi) JEV positive human serum and (vii) Dengue antigen. [Inset shows the Randle Equivalent circuit]	71
Figure 4.6	Bar chart of (i) bare SPCE strip; (ii) CNPs modified SPCE strip; (iii) JEV antibody immobilized CNPs on SPCE strip; (iv) BSA blocked/JEV antibody immobilized CNPs on SPCE strip; (v) BSA blocked/JEV antibody immobilized CNPs on SPCE strip in the presence of JEV; (vi) JEV positive human serum and (vii) Dengue antigen.	72
Figure 4.7	(A) Nyquist plots of JEV antibody immobilized CNPs on SPCE strip with different JEV concentrations (ng/mL), (B) show calibration plots of Relative resistance versus concentrations of JEV ranging from 1 to 20 ngmL ⁻¹ and Inset of (A) show the Randle Equivalent Circuit. (Error bars of inset of (B) were calculated from the mean value, s/n = 3).	74
Figure 4.8	(A) Cyclic voltammograms of JEV antibody immobilized CNPs on SPCE biosensor strip as a function of response time: (a) 0 min; (b) 5 min; (c) 10 min, and (d) 15 min and (B) shows the plot of current (μA) versus response time (min).	76

Figure 5.1	Schematic diagram of preparation processes employed on the SPCE strip for fabricating electrochemical biosensor for the detection of JEV (Lai et al., 2017).	84
Figure 5.2	UV-Vis absorption spectra of the chitosan carbon nanoparticles (CNPs), colloidal gold nanoparticles solution (AuNPs), hybrid 10 nm gold-carbon nanoparticles (Au10-CNPs), hybrid 20 nm gold-carbon nanoparticles (Au20-CNPs) and hybrid 40 nm gold-carbon nanoparticles (Au40-CNPs)	86
Figure 5.3	Transmission electron micrographs of pure colloidal gold nanoparticles (AuNPs) of (A) 10 nm AuNPs, (B) 20 nm AuNPs, (C) 40 nm AuNPs; and hybrid gold-carbon nanoparticles (Au-CNPs) of (D) Au10-CNPs, (E) Au20-CNPs and (F) Au40-CNPs	87
Figure 5.4	Transmission electron micrographs (TEM) of hybrid 10 nm gold-carbon nanoparticles (Au10-CNPs)	88
Figure 5.5	FESEM (left) images and energy dispersive X-ray (EDX) spectrum (right) of hybrid gold-carbon nanoparticles for (A) Au10-CNPs, (B) Au20-CNPs and (C) Au40-CNPs	89
Figure 5.6	CV of bare SPCE strip, CNPs SPCE strip, Au10-CNPs on SPCE strip, Au20-CNPs on SPCE strip, Au40-CNPs on SPCE strip.	90
Figure 5.7	Nyquist plots of bare SPCE strip, CNPs SPCE strip, Au10-CNPs on SPCE strip, Au20-CNPs on SPCE strip, Au40-CNPs on SPCE strip. [Inset shows the Randle equivalent circuit]	92
Figure 5.8	(A) Nyquist plots of ratio AuNPs:CNPs for 1:1; 2:1; 3:1; 4:1; 5:1; 6:1; 7:1; 8:1; 9:1 and 10:1 (B) Error bar shows electron transfer resistance, R_{et} for various Nyquist plot of Ratio AuNPs:CNPs (1:1; 2:1; 3:1; 4:1; 5:1; 6:1; 7:1; 8:1; 9:1 and 10:1). [Inset of (A) shows the Randle Equivalent circuit]	94
Figure 5.9	Nyquist plots of various electrolyte temperature of 10 °C, 20 °C, 35 °C, 45 °C, 60 °C and 80 °C.	95
Figure 5.10	(A) Cyclic voltammograms of JEV antibody immobilized Au10-CNPs on SPCE biosensor strip as a function of response time: 1 min, 3 min, 5 min, 7 min, 10 min, 13 min, 15 min and 20 min and (B) Plot of current (μ A) versus response time (min).	96
Figure 5.11	Nyquist plots of (i) Au10-CNPs SPCE strip; (ii) Au10-CNPs + JEV Ab on SPCE strip; (iii) Au10-CNPs + JEV Ab + BSA on SPCE strip; (iv) Au10-CNPs + JEV Ab + BSA + Dengue on SPCE strip; (v) Au10-CNPs + JEV Ab + BSA + Positive Serum [Inset (left) Chart shows electron transfer resistance, R_{et} for various Nyquist plot (i-v)]; [Inset (right) shows the Randle Equivalent circuit]	97

- Figure 5.12 Nyquist plots of JEV antibody immobilized Au10-CNPs on SPCE strip with different JEV concentrations (ng/mL), Inset shows the Randle Equivalent Circuit. 99
- Figure 5.13 Line graph show calibration plots of relative resistance versus concentrations of JEV ranging from 1 to 20 ngmL⁻¹ with R²=0.999 which were calculated from the mean value, s/n = 3. 99

LIST OF ABBREVIATIONS

Au-CNPs	Hybrid Gold-Carbon nanoparticles
Au10-CNPs	Hybrid 10 nm Gold-Carbon nanoparticles
Au20-CNPs	Hybrid 20 nm Gold-Carbon nanoparticles
Au40-CNPs	Hybrid 40nm Gold-Carbon nanoparticles
Au-CNPs-SPCE	Hybrid Gold-Carbon nanoparticles modified on screen-printed carbon electrode
BSA	Bovine serum albumin
CNs	Carbon nanomaterials
CNTs	Carbon nanotubes
CNPs	Carbon nanoparticles
CNPs-SPCE	Carbon nanoparticles modified on screen-printed carbon electrode
CV	Cyclic voltammetry
DNA	Deoxyribonucleic acid
EDC	N-(3-Dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride
EIS	Electrochemical Impedance Spectroscopy
FTIR	Fourier Transform Infrared Spectroscopy
GCE	Glassy carbon electrode
GO	Graphene oxide
JE	Japanese encephalitis
JEV	Japanese encephalitis virus
LOD	Limit of detection
MAC-DOT	IgM capture dot enzyme immunoassay

MAC-ELISA	IgM antibody capture enzyme-linked immunosorbent assay
MW-CNT	Multiple wall carbon nanotube
NDs	Nanodiamonds
NHS	N-Hydroxysuccinimide
PBS	Phosphate buffer saline
PRNT	Plaque reduction neutralization test
R_{et}	Electron-transfer resistance
RNA	Ribonucleic acid
RT-PCR	Reverse transcription polymerase chain reaction
SAM	Self-assembly method
SCE	Standard calomel electrode
SEM	Scanning electron microscopy
SHE	Standard hydrogen electrode
SPCE	Screen-printed carbon electrode
SW-CNT	Single wall carbon nanotube
UNIMAS	Universiti Malaysia Sarawak
UV-Vis	Ultraviolet visible Spectroscopy
WHO	World Health Organisation

CHAPTER 1

INTRODUCTION

1.1 Background and Problem Statement

Japanese encephalitis virus (JEV), a mosquito-transmitted flavivirus consisting of a positive-sense single-stranded ribonucleic acid (RNA) genome which acts as messenger RNA encoding a single open reading frame (Yun & Lee, 2014). This will then lead to neurological diseases and death in humans in extreme cases (Li et al., 2015; Bharucha et al., 2018). World Health Organisation (WHO) has reported that JEV outbreaks first happened in Japan during the 19th century. In recent years, JEV has become a major public health concern globally as there are estimated 70,000 human cases and 20,000 deaths annually (Nain et al., 2016). Mansfield et al. (2017) pointed out some reproductive problems such as abortion, still-birth and birth defects happening to adults infected by JEV. On the other hand, JE is the major cause of encephalitis and 75% happened to children less than 15 years old in Asia (Mansfield et al., 2017). According to Zanin et al. (2003), vaccination for JEV has been available and used internationally since the 1930s and the commercially used vaccines now in the market are JE-VAX and JE-VC, respectively. However, Mansfield et al. (2017) reported that hypersensitivity and licensed reason in certain countries are some of the reasons for the uncontrollable outbreaks of JEV in endemic countries. Accurate diagnose is therefore important to prevent and control the JEV outbreaks.

Some conventional diagnostic devices have been used for the diagnosis of JEV infection, for example, enzyme-linked immunosorbent assay (ELISAs) method, plaque reduction neutralization test, reverse transcription-polymerase chain reaction (RT-PCR) and virus isolation (Hobson-Peters, 2012). These methods involve costly equipment and

specialized expertise to run the laborious and slow analyses. A simple nitrocellulose membrane-based immunoglobulin M (IgM) capture dot enzyme immunoassay (MAC DOT) was developed and commercialized for the diagnosis of JE (Solomon et al., 1998). The IgM antibody capture (MAC ELISA) is the first-line serological assay recommended by the World Health Organization (WHO) for JE diagnosis. There are several types of MAC ELISA kits currently available commercially. However, studies conducted on three of the most common MAC ELISA kits (Panbio JE-Dengue IgM combo ELISA, XCyton JEV CheX and InBios JE Detect) have shown that they have low sensitivities which ranged between 17% and 57% (Robinson et al., 2010). Furthermore, all these diagnostic methods are confined to qualitative analysis only.

Majority of the JE cases happened in rural areas in Southern and Eastern Asian Countries, where access to diagnostic laboratory facilities is very limited. Mansfield et al. (2017) mentioned JEV infection is difficult to be detected due to a short duration of viremia. Hence, a portable biosensor system that can provide point-of-care, rapid, sensitive and economical diagnostic tool is highly needed for such settings. Undeniably, it has drawn the attention of many researchers in the related field to explore and design different configurations and approaches to preparing biosensors. This is important as many dangerous bacterial infections could be triggered by as low as 10 organisms. Various configurations of biosensors are being explored recently which includes antibody-based system, enzyme-based detection, and DNA-based sensors. The biosensor has proven its high potential in terms of ability to shorten the detection time from 2 to 4 days to less than an hour *via* a simple target extraction method (Wang & Dai, 2015). This is an important discovery in the medicinal field which shows integration in ensuring a fast and easy way to obtain a signal from biological events (Liu & Guo, 2012).